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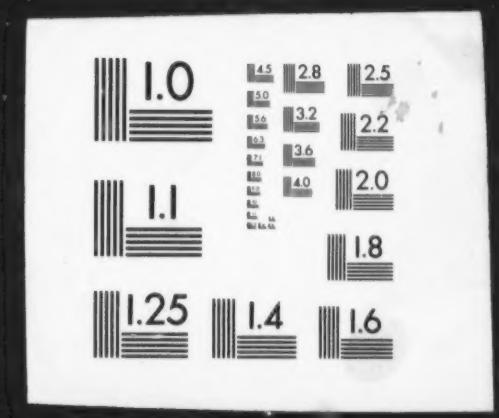

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Liquid and Gas Chromatographic Analysis of Diethyl Phthalate in Water and Sediment

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16. ABSTRACT

Diethyl phthalate was determined in water and sediment by high performance liquid chromatography (HPLC) and in water by gas-liquid chromatography with electron capture detection (GLC-ECD). Water samples were extracted with hexane, using a high-speed homogenizer-ultrasonic apparatus and a test tube mixer. Sediments were Soxhlet-extracted using acetonitrile. For HPLC, diethyl phthalate was determined in normal phase mode using a Zorbax-CN column, a 2 % isoproponol-hexane solvent system, and a UV variable wavelength detector. For GLC-ECD, a 3 % SE-30 Gas-Chrom Q column with a 63-Ni electron capture detection system was used. Recoveries from fortified damples ranged from 93.9 to 98.0 % for water at 0.01-0.50 ppm, and from 90.0 to 93.6 % for sediment at 0.2-2.0 ppm.

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Liquid and Gas Chromatographic Analysis of Diethyl Phthalate in Water and Sediment

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Diethyl phthalate was determined in water and sediment by high performance liquid chromatography (HPLC) and in water by gas-liquid chromatography with electron capture detection (GLC-ECD). Water samples were extracted with hexane, using a high-speed homogenizer-ultrasonic apparatus and a test tube mixer. Sediments were Soxhlet-extracted using acetonitrile. For HPLC, diethyl phthalate was determined in normal phase mode using a Zorbax-CN column, a 2% isopropanol-hexane solvent system, and a UV variable wavelength detector. For GLC-ECD, a 3% SE-30 Gas-Chrom Q column with a ^{63}Ni electron capture detection system was used. Recoveries from fortified samples ranged from 93.9 to 98.0% for water at 0.01–0.50 ppm, and from 90.0 to 93.6% for sediment at 0.2–2.0 ppm.

The EXposure Analysis Modeling System (EXAMS), a computerized system for analyzing the fate of toxic substances in aquatic environments, is currently under development by the Environmental Protection Agency (L. Burns and M. Cline, EPA, Athens, GA). Tests to compare predictions from EXAMS with experimental data from controlled laboratory ecosystems required an analytical method for diethyl phthalate (DEP) in water and sediment. The research dictated that the method be applicable to large numbers of samples with emphasis on reducing the time between sampling and extraction procedures.

DEP is widely used as an insect repellent, for plasticizing cellulose esters, in propellant formulations, and in the manufacture of fragrances. The production of phthalate esters in the United States has increased at a steady rate from 300 million pounds in 1960 to over 1 billion pounds in 1975. The annual production is expected to increase to approximately 10 billion pounds by the turn of the century (1).

Peakall (2) reviewed the occurrence, biological activity, toxicology, and accumulation factors for phthalates in the environment. The review pointed out that data are lacking to adequately assess the environmental risk and to predict the point of occurrence of maximum residue levels.

Several authors have addressed the analytical procedures and problems in the analysis of phthalates. Fishbein and Albron (3) reviewed the analytical methods, biological properties, stability, and episodes of phthalate occurrence in the enviornment. Giam et al. (4–6) and Ishida et al. (7) considered the problem of background contamination from phthalates present in commercial laboratory reagents, glassware, and equipment.

The use of high performance liquid chromatography (HPLC) was proposed by Mori (8) for the determination of phthalates in river water and by Persiani and Cukor (9) for the determination of phthalates in industrial effluents and biological samples.

Corcoran (10), Thomas (11), and Norwitz and Apatoff (12) proposed gas chromatographic (GLC) methods using the electron capture detector (ECD) for phthalates. The detector, which responds to many classes of compounds with an affinity for electrons, usually exhibits high background because of interference from co-extractives in environmental samples with inadequate or no cleanup. Time-consuming cleanup and isolation techniques cannot be omitted in many samples, especially sediments, if the electron capture detector is to be used for determination. The problem of background interferences can be lessened by the use of HPLC. Less sample preparation and cleanup is required by taking advantage of the wide selection of column types, mobile phases, and detectors available, such as the variable wavelength UV detector. For example, the molar absorption coefficient at 224 nm, the maximum absorption wavelength for DEP, is approximately 10 times that of the standard fixed wavelength, 254 nm, thus providing some degree of selectivity for this detector.

METHOD

Apparatus and Reagents

(a) *High performance liquid chromatograph.*—Tracor Model 900-78S chromatograph with a

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970A variable wavelength UV detector and Valco UCLI closed loop injection system. Column was Zorbax-CN (E. I. du Pont de Nemours & Co., Inc., Wilmington, DE), 25 cm × 4.6 mm id, with Whatman Co:Pell PAC guard column and mobile phase of 2% isopropanol–hexane at 1.0 mL/min and 350–400 psi.

(b) *Gas-liquid chromatograph.*—Tracor Model 222Q chromatograph with ^{63}Ni electron capture detector operated in the DC mode. Column was borosilicate glass, 8 ft × 1/4 in. od cut for off-column injection and packed with 3% SE-30 on 80–100 mesh Gas-Chrom Q. Operating conditions: nitrogen flow rate, column 50 mL/min, purge 30 mL/min; temperatures (°C)—column, 200; detector, 275; injection port, 240; transfer line, 275; Westronics MT-22 recorder 30 in./h.

(c) *Homogenizer.*—Brinkmann Polytron PT-10-35 with PT-20ST probe generator.

(d) *Test tube mixer.*—Vortex Genie K-550-G.

(e) *Soxhlet apparatus.*—Pyrex 3840.

(f) *Solvents.*—Acetonitrile, hexane, isooctane, isopropanol—distilled in glass, all solvents HPLC grade (Burdick & Jackson Laboratories, Inc., Muskegon, MI).

(g) *Diethyl phthalate.*—(Aldrich Chemical Co., Milwaukee, WI). Weigh 50 mg and place into 100 mL volumetric ⊽ flask and dilute to volume with final extracting solvent. Stock standards are stable if stoppered tightly, sealed, and protected from light under refrigeration.

HPLC Determination

(a) *Water.*—Transfer 300.0 mL sample to 500 mL reagent bottle that contains 100 mL hexane. Extract samples, using Polytron homogenizer 2 min at 25 000 rpm. Transport bottles to laboratory for following additional extractions: Transfer contents of bottles to 1 L separatory funnels with hexane, allow phase separation, and transfer water layer to second 1 L separatory funnel. Collect hexane from first extraction in 400 mL beaker. Extract water in 1 L separatory funnel two more times, using homogenizer and 100 mL and 50 mL portions of hexane. Add these extracts to first extract in 400 mL beaker and concentrate combined hexane extracts on water bath to ca 5–8 mL. Transfer quantitatively with hexane to graduated (calibrated) 15 mL conical test tubes and seal with ⊽ stoppers.

(b) *Sediment.*—Clean glass wool and filter papers (Whatman No. 42, 15 cm) by placing them in large Soxhlets and extracting 4 h with hexane. Transfer glass wool and filter papers to beakers and place on steam bath under hood to evaporate hexane. Spread filter papers on acetone-rinsed aluminum foil and dry 1 h in 120°C oven. Place in desiccator, cool 5 min, remove one at a time, number, and record tare weight. Place Büchner filtering funnel (127 mm od) on side-arm filtering flask with filter-vac disc. Prewet filter paper and shape into Büchner funnel, leaving small rim of filter paper turned up around sides of funnel. Seat paper with low vacuum, add wet sediment, and rinse with ca 10 mL distilled water to form sediment pad. Release vacuum, remove pad and filter, and gently fold in fluted form. Place sediment and filter into Soxhlet extractor containing small plug of glass wool at the bottom and add small plug of glass wool to top of sample. Extract 4 h with acetonitrile. Siphon over any acetonitrile remaining in upper Soxhlet after extraction and rinse and siphon several times, collecting siphonings in beaker. Remove top glass wool plug, place pad in small beaker, and dry overnight in 120°C oven. Remove from oven to desiccator, cool for 30 min, weigh pad, and subtract tare of filter paper to obtain total net dry weight.

Quantitatively transfer acetonitrile extract to beaker containing previous rinses and concentrate to ca 100 mL. Transfer quantitatively to 2 L separatory funnel containing a 10× dilution of distilled water. Extract 3 times with 100, 100, and 50 mL portions of hexane, collecting all extracts in 400 mL beaker. Concentrate combined extracts on water bath to 5–8 mL, transfer quantitatively to 15 mL graduated (calibrated) conical test tube, and seal with ⊽ stopper. Record volume in graduated tubes for water and sediment extracts. Mix thoroughly and, using 50 μL syringe, inject into 20 μL HPLC sample loop. DEP concentration is determined by peak height comparison of sample and DEP reference standard.

GLC-ECD Determination for Water

Transfer 2.0 mL sample to 15 mL ⊽ conical test tube and add 1.0 mL isooctane. Experimental data on 6 sample:solvent ratios show that sample size may be varied with good toxicant recovery if the sample:solvent ratio is 2:1. Extract by mixing stoppered sample tubes 2 min on vortex mixer at 1000–1200 rpm. Let solvent-water layer separate and inject 3–8 μL of top isooctane layer into gas chromatograph. DEP concentration is determined by comparing peak heights of sample with DEP standard.

Results and Discussion

Fortified ecosystem water samples were chromatographed on a Zorbax-ODS methanol–

Table 1. Recoveries of DEP from fortified samples of water and sediment

Substrate	Amt added, ppm	No. of analyses	Recovery, %
Water (300 mL) HPLC	0.01	7	94.6 ± 1.6
	0.02	7	93.9 ± 3.8
	0.05	6	94.8 ± 2.1
	0.10	14	96.3 ± 2.7
Sediment (20.0 g) HPLC	0.2	4	93.6 ± 2.8
	0.4	4	90.0 ± 4.3
	0.8	4	90.6 ± 2.8
	2.0	12	92.5 ± 1.8
Water (2.0 mL) GLC-ECD	0.02	6	94.9 ± 2.1
	0.05	6	94.6 ± 2.3
	0.10	11	98.0 ± 2.3
	0.50	6	96.4 ± 2.2

water reverse phase HPLC system to explore the feasibility of direct sample injection. The lower limit of detection for this system was in the range of 1.50–0.50 ppm, which was higher than the anticipated concentration of DEP in the model ecosystem.

To concentrate the toxicant for HPLC, several solvents were tested for liquid-liquid extraction. Analytical results showed that a DEP-solvent mixture could not be evaporated completely to dryness and redissolved in methanol for the reverse phase system without subsequent loss of toxicant. Solutions of DEP in methanol, hexane, and methylene chloride evaporated on a water or dry bath at 80°C to 0.5–1.0 mL, transferred to conical test tubes, and evaporated to dryness with a stream of dry air, showed losses ranging from 10 to 25%. Hexane, which was selected as the extracting solvent, was concentrated to 5–8 mL and transferred to a 15 mL graduated ☤ centrifuge tube for HPLC determination.

Several HPLC systems compatible with hexane were investigated and a normal phase Zorbax-CN column, using isopropanol hexane mobile phase, was selected. The detection limit for HPLC using this system was 20 ng DEP at 25% full scale deflection (FSD). The recovery from water samples fortified with DEP-distilled water standards is shown in Table 1. Figure 1 demonstrates the response of DEP at 224 nm compared with that at 254 nm, and chromatograms of DEP in ecosystem samples are shown in Figure 2.

HPLC was the method of choice because it provided a technique for DEP in water and sediment with little or no cleanup. But for water analysis, a minimum sample volume of 300 mL was required to achieve method sensitivity. The GC-ECD method was used as a confirming technique for DEP in water, and this simple, rapid method would be applicable to situations where sample volume is limited. No major difficulty was experienced in the evaluation of the resulting chromatograms (Figure 3). However,

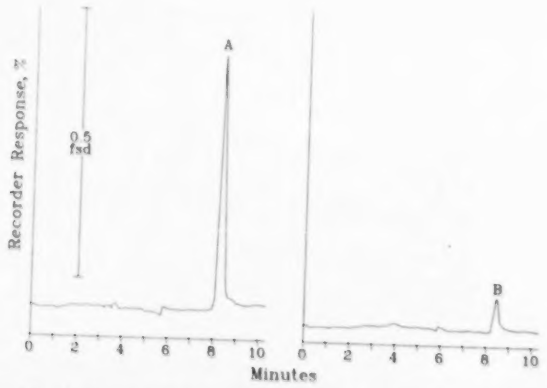

Figure 1. Liquid chromatograms of DEP response at 224 nm (A) and 254 nm (B).

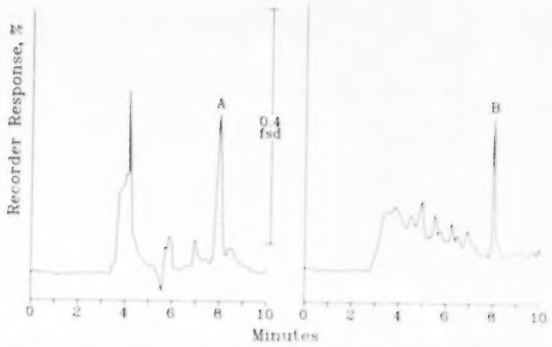

Figure 2. Liquid chromatograms of DEP in ecosystem water at 0.05 ppm (A) and sediment at 2.0 ppm (B).

it is probable that some environmental water samples would require cleanup using techniques such as the deactivated (3% water) Florisil procedure described by Giam et al. (4, 6). The detection limit for the ECD was 0.5 ng DEP at 20% FSD and recovery from fortified small volume water samples is shown in Table 1. Attempts to analyze the sediment extracts by ECD after acetonitrile–hexane partitioning were not successful, and the main disadvantage of this method is that many environmental samples (e.g., large water volumes, sediments) must undergo rigorous cleanup before ECD determination. The continuous injection of samples with inadequate cleanup can contaminate the GLC column and the EC detector, causing tailing, baseline noise, drift, and loss of sensitivity. However, the potential application of this method to water samples from small model ecosystems as a screening and/or confirming technique for DEP should not be overlooked.

To minimize the problem of interferences from other phthalates such as diethyl hexyl phthalate (DEHP), a common laboratory contaminant, glassware, filters, and reagents were cleaned and extracted with high purity solvents and/or heat-treated (6). As a result, procedural blanks did not show any significant background which interfered with DEP. However, the ability of this HPLC system to resolve DEP and DEHP is illustrated in Figure 4.

Distilled water standards of DEP should be kept under refrigeration when not in use. DEP-water standard held at ambient temperature

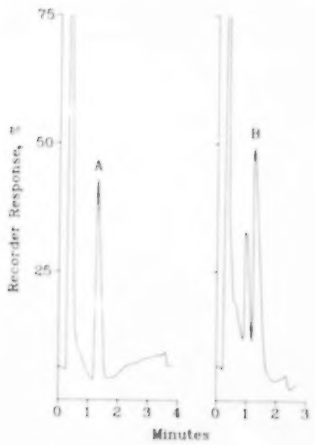

Figure 3. Gas chromatograms of 2.0 ng DEP standard (A) and DEP in ecosystem water at 100 ppb (B).

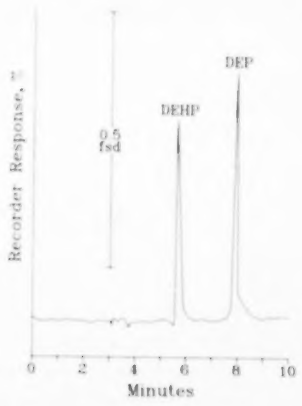

Figure 4. Liquid chromatograms of standard mixture containing DEHP (100 ng) and DEP (100 ng) in hexane at 224 nm.

under laboratory fluorescent lighting decreased in concentration by 20% in 4 days and 70% in 12 days. No DEP could be detected after 14 days. DEP loss in distilled water in the absence of light was somewhat slower. Water standards of DEP held under refrigeration showed no loss after 60 days.

The water samples for HPLC determinations were extracted on-site immediately after sampling, and the GLC water samples were refrigerated and extracted within 1–2 h after sampling. There were no significant differences in the final results. However, the need to minimize the lag time from sampling to extraction was demonstrated when a water sample from the ecosystem was analyzed, held at ambient temperature, and re-analyzed in 24 and 48 h. These re-analyses revealed DEP losses of 64 and 89%, respectively, from the initial concentration. When it is not possible to extract the samples promptly, the need for immediate refrigeration cannot be overemphasized. Selected stored samples should be reanalyzed at intervals and fortified control samples should be held under the same conditions to determine the effects of cold storage.

The acetonitrile-hexane partitioning procedure provided adequate cleanup for HPLC determination in all sediment samples analyzed. The recovery from sediment samples fortified with DEP-distilled water standards is shown in Table 1. If time is not a factor, additional cleanup is recommended in order to prolong the life of the HPLC column, and detector cell windows.

The use of the Polytron ultrasonic apparatus for the extraction of DEP from sediments was also investigated and the difference in recoveries between the Polytron and Soxhlet techniques was small. The ultrasonic procedure is much faster than the Soxhlet but the stainless steel generator probe wears rapidly because of the abrasive action of the sediment. Johnsen and Starr using this apparatus for soil extractions reported that replacement was necessary after 150–200 extractions (13). The current replacement cost is $600. The Soxhlet technique, although lengthy, requires minimum technician attendance after the initial set up until the extraction period is completed.

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